Abstract

Harnessing the immune system to eliminate cancer and prevent its recurrence is proving to be a powerful therapeutic approach that has achieved unprecedented successes in hematological malignancies and some solid malignancies with a high tumor burden. Next generation immune therapies to improve clinical responses and widen the reach of immunotherapy are being designed at a rapid pace, and canine cancer patients offer an under-utilized opportunity to provide information on safety, efficacy and mechanisms of these therapies, because dogs are immunologically outbred, immune competent and develop spontaneous cancers such as non-Hodgkin’s lymphoma, glioblastoma, osteosarcoma, urothelial carcinoma and melanoma that share remarkable clinical, biological and genetic features with human counterparts. As such, pre-clinical testing of immune therapeutic approaches in dogs with cancer promises to inform the development of immunotherapies in human cancer patients.

The Cancer IO Panel has been uniquely designed with 800 canine genes for pan-cancer immune response studies. The panel represents a companion to the widely recognized nCounter Human IO 360® and Human PanCancer Immune Profiling panel used in human clinical trials, with significant overlapping content designed for directly comparing human and canine immune responses. The customizable panel segments genes into 8 core components including Cytokine & Chemokine Signaling, Interferon Signaling, Checkpoint Signaling, Complement Cascade, Immune Cell Abundance, Tumor Immunogenicity, Inhibitory Tumor Mechanisms, and Stress Factors. Genes were selected based on their relevance for the study of oncology, their importance in human clinical studies as well as canine expression profiles from both RNA-Seq and nCounter experiments. Additionally, the canine reference transcriptome, based on CanFam3.1, was utilized for designing the probes; the known genomic variability of dogs in addition to the nCounter hybridization chemistry result in compatibility across a variety of species and veterinary studies aiming to develop and improve the understanding and treatment of both canine and human cancers.

Comparison of nCounter versus RNA-Seq for Canine Immune Profiling

The era of effective cancer immunotherapy represents a major change in how cancer is treated, and canine cancer patients undoubtedly have an opportunity to play an important role in advancing this field. The value of using canine cancer patients as a pre-clinical model for immunotherapy has been demonstrated previously, with the best example being the essential role played by dogs with osteosarcoma in the development of the non-specific immunotherapeutic L-MTP (liposomal muramyl tripeptide) as an approved immunotherapy for pediatric osteosarcoma. Immune competent dogs with spontaneous cancers offer an underutilized opportunity to provide information on safety, efficacy and correlative biomarkers of response to next generation immunotherapies – accelerating their translation into human trials. Development of comparable tools for deep immune profiling of the canine immune response, here we present work conducted through a year-long, multi-center, global collaboration resulting in the creation of a novel gene expression tool for studies of the immune response in dogs treated with immune therapy and targeted therapies. This approach combines NanoString’s nCounter® platform with the results can be translated promptly to benefit both dogs and humans, with their shared tumor types and strong bonds.

Comparative Canine Oncology

In the first, a Nanostring panel of 305 genes was applied to 13 cases of CD8+ T zone lymphoma and CD8+ T cells purified from the lymph nodes of 5 healthy dogs. The log2 fold change in gene expression between the averages of cases and controls was determined and each gene plotted on the X axis. In a separate RNA-seq experiment using 7 different cases of CD8+ T zone lymphoma and CD8+ T cell controls, the log2 fold change between cases and controls was calculated for the genes used in the first Nanostring study y-axis, R2 between the two methods is .332, p < .001. The results with the two gene panels were similar with the same subset of 10 genes for immune cell enrichment. The RNA-seq data were interrogated using a published list of genes that classify human cancer as T cell infiltrated (enriched genes indicated in red) or non-T cell infiltrated defined by Swis et al. Cancer Immunol Res, 2016 (B), and using the genes in the canine IO panel (C). Each column represents data from one case. Note that the results with the two gene panels were similar with the same subset of 10 cases (see right side of both heatmaps) being especially T cell infiltrated.

Efficient Transition from RNA-Seq to nCounter for Canine Translational Oncology

Figure A Comparison of gene expression between T zone lymphoma and normal CD8+ T cells. Two separate experiments using RNA from different cases and controls are shown in the first, a Nanostring panel of 305 genes was applied to 13 cases of CD8+ T zone lymphoma and CD8+ T cells purified from the lymph nodes of 5 healthy dogs. The log2 fold change in gene expression between the averages of cases and controls was determined and each gene plotted on the X axis. In a separate RNA-seq experiment using 7 different cases of CD8+ T zone lymphoma and 3 CD8+ T cell controls, the log2 fold change between cases and controls was calculated for the genes used in the first Nanostring study y-axis, R2 between the two methods is .332, p < .001.

Figure B and C Interrogation of RNA-seq data from 29 canine invasive urothelial carcinomas (nCUC) for immune cell enrichment. The RNA-seq data were interrogated using a published list of genes that classify human bladder cancer as T cell infiltrated (enriched genes indicated in red) or non-T cell infiltrated defined by Swis et al. Cancer Immunol Res, 2016 (B), and using the genes in the canine IO panel (C). Each column represents data from one case. Note that the results with the two gene panels were similar with the same subset of 10 cases (see right side of both heatmaps) being especially T cell infiltrated.

Conclusions and Next Steps

The era of effective cancer immunotherapy represents a major change in how cancer is treated, and canine cancer patients undoubtedly have an opportunity to play an important role in advancing this field. The value of using canine cancer patients as a pre-clinical model for immunotherapy has been demonstrated previously, with the best example being the essential role played by dogs with osteosarcoma in the development of the non-specific immunotherapeutic L-MTP (liposomal muramyl tripeptide) as an approved immunotherapy for pediatric osteosarcoma. Immune competent dogs with spontaneous cancers offer an underutilized opportunity to provide information on safety, efficacy and correlative biomarkers of response to next generation immunotherapies – accelerating their translation into human trials. Development of comparable tools for deep immune profiling of the canine immune system using the nCounter® technology is critical for translating results from preclinical studies in dogs to clinical application in humans. This is especially true in situations where essential reagents must be shared or where access to patients with certain tumor types is limited. The best possible outcomes will be studies where the results can be translated promptly to benefit both dogs and humans, with their shared tumor types and strong bonds.

Acknowledgements

The authors would like to thank the National Cancer Institute Cancer Moonshot Program for their support of the Precision Consortium and Canine Comparative Oncology studies.